*INQUILINIC PROTOZOA FROM FRESHWATER GASTROPODS. II. *CALLIMASTIX JOLEPSI* N. SP., FROM THE INTESTINE OF THE PULMONATE FRESHWATER SNAIL, *HELISOMA DURYI* SAY, IN FLORIDA

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The genus *Callimastix* was originated by Weissenberg (1912) for a parasitic, polymastigote protozoan which he found in the hemolymph of the copepod crustacean, *Cyclops strenuus.* He believed the genus to be related to *Lophomonas;* and he named it *Callimastix cyclopis.* Recently, Vavra (1960) has reported the same species from related copepods, *Mesocyclops leuckartii* and *Megacyclops viridis.* It has not otherwise been reported from other invertebrates. Other species, from the intestinal tracts of herbivorous mammals, have been reported as: (1) *Callimastix frontalis* from ruminants (Braune, 1913), from ruminants in South America (da Fonseca, 1915), from cattle (Becker and Talbott, 1927), and from the Indian goat (Das-Gupta, 1935); and (2) *Callimastix equi,* from the horse (Hsiung, 1929, 1930). The latter species is considered by some to be identical with *C. frontalis* (Grassé, 1953), and distinct by others (Kudo, 1954; Hall, 1953).

Da Fonseca (1915) set up the family *Callimastigidae* for the genus. The family is recognized by Hall (*loc. cit*). and Kudo (*loc. cit.*) as resident in the polymastigote order of zooflagellates. It is also recognized by Reichenow (1952), but only as appendant to the family *Hypermastigidae* in the polymastigote order. The family is also mentioned by Grassé (*loc. cit*), but he does not recognize it, and he does not place the genus in any recognized affinity with any other family or order of zooflagellates.

During November of 1959, my attention was directed to a polymastigote flagellate from the intestine of a specimen of the freshwater, pulmonate snail, *Helisoma duryi* Say. I identified it as a species of *Callimastix.* Further study of numerous specimens of the protozoan from other individual snails of the indicated species

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showed that it differs morphologically, as well as in the host which supports it, from *C. cyclopis, C. frontalis,* and *C. equi.* I propose that it be called *Callimastix jolepsi* n. sp., hereinafter described.[1]

Materials and Methods

About fifty snails of the species *Helisoma duryi* were collected during late November, and in December 1959, and in January 1960, from a shallow surface pond (Grove Hall Pond) on the campus of the University of Florida at Gainesville, Florida. The snails were kept in a laboratory at about 20° to 22° C, in a loosely covered quart glass jar which contained water from the pond, green and blue-green algae, a variety of vegetable detritus, and an association of planktonic algae, protozoa and microcrustacea.

The intestinal contents of each snail was expelled into a separate small dish containing 10 to 20 cc of an invertebrate saline (Neff, 1957). Aliquots (0.5 ml) were pipetted, each to a clean microscope slide, and covered with a coverslip. The area thus prepared was scanned for the presence of callimastigid protozoa. Five snails were found to be infected, one very heavily so.

Microscopic observations were made on living organisms, and of organisms fixed in formalin, but not stained. Both bright-field and variable-phase-contrast interferometric microscopy were used at 100X to 1000X magnifications. A standard research-type microscope lamp, set for Köhler illumination, provided light. Filters employed were "daylight" blue, sodium-green, ground-glass, and heat-absorbent glass, singly and in combinations.

Measurements were made at 400X under interferometric, dark-phase-contrast illumination, with sodium-green filter, by means of a calibrated ocular micrometer.

About 150 organisms were examined, 125 of them critically.

Observations

Size and Shape: Most of the individual protozoans were between 15 and 18 μ long, a few as short as 11 μ, and one was 20 μ long. The broad diameter varied from 12 to 16 μ; and the nar-

[1] The specific name for this protozoan is proposed in grateful acknowledgement to Mrs. Josephine Leps Patterson who brought this organism to my attention during her studies on some protozoan associates of aquatic invertebrates for a senior biology major research problem.

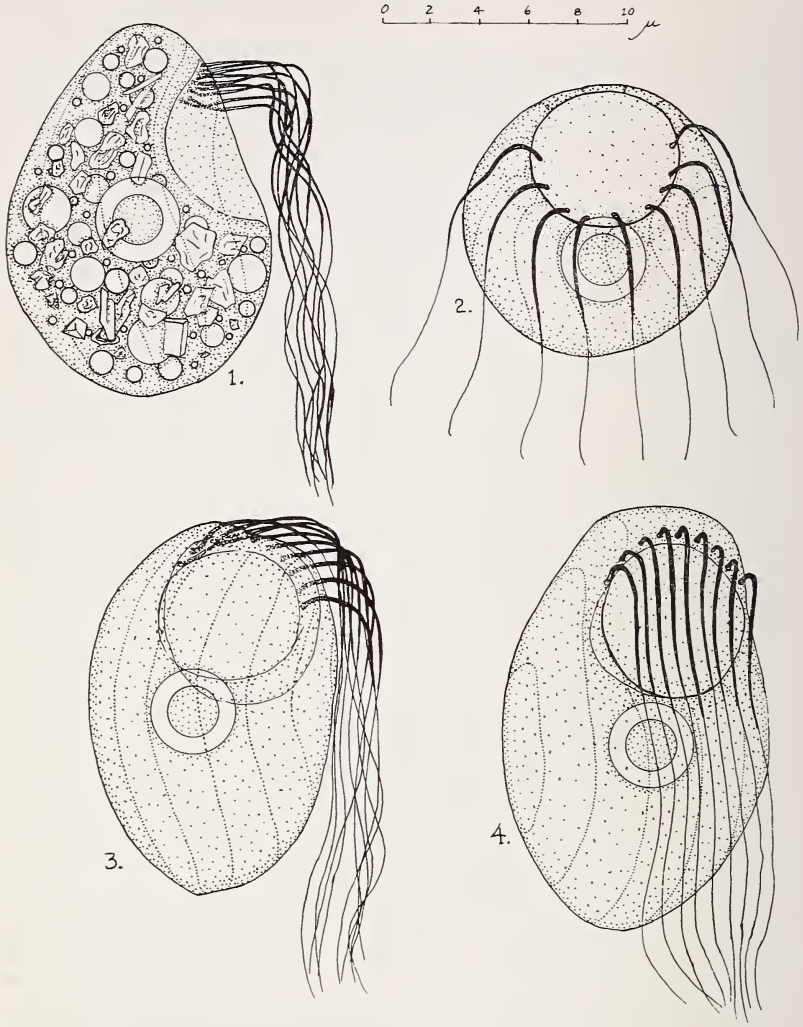

Fig. 1. Lateral view of the organism's right aspect, showing the cup-shaped depression and apical cilia at the upper right. The nucleus is the large spherical object in the center of the body. Crystals, amorphous bodies, globules and granules are shown as inclusions. Striations of the pellicle are shown in dotted lines.

Fig. 2. An apical view of a living organism showing the position of the flagella just before being snapped forward over the cup-shaped depression in the rhythmic beat of the flagella.

Fig. 3. A postero-lateral view of the organism.

Fig. 4. An antero-lateral view of the organism looking towards the cup-shaped depression.

Inclusions other than the nucleus are omitted from Figs. 2, 3, and 4.

rower diameter from 10 to 13 μ. The shape is egg-like from one lateral view (Fig. 1), and broadly oval from the aspect 90° rotated (Fig. 4). The contour viewed polarly is spherical to elliptical (Fig. 2). At the flagellar pole there is a cup-shaped depression on one broader surface, the apical rim of which bears the flagella (Figs. 1-4).

Flagella: These organelles extend from separate kinetosomes at the apical rim of the cup-shaped depression, appearing as a tangled brush in fixed specimens, but beating in well-coordinated unison in the living individual (Figs. 1-4). There were no fewer than 7 and no more than 11 flagella on any of the specimens studied. The majority of the organisms observed had 9 or 10 flagella. On live specimens each flagellum measured 25 to 30 μ long (the longer ones on the larger organisms), were about 0.25 μ in diameter at the bases, with a distinct, slightly swollen kinetosome at the base of each, tapering regularly to the tips which were barely resolvable. The flagella of the fixed organisms were somewhat contracted measuring 18 to 26 μ long, and about 0.3 thick at the bases and about 0.25 μ at the tips.

Nucleus: This organelle is globular, with a distinct endosome which is still more distinct in fixed specimens (Fig. 1). In the living protozoan the nucleus is about 3 μ diameter ($\pm$ 0.35 μ); and the endosome about 2 μ diameter ($\pm$ 0.25 μ). In fixed specimens both nucleus and endosome are slightly smaller in diameter.

Contractile Vacuole: None is present.

Other Inclusions: Irregular granules, 1.2 to 2.7 μ long, and highly refractile, are abundant in the cytoplasm, except in the thin zones beneath the cup-shaped depression. Other barely identifiable, barely resolvable granules are also present; as well as small refractile globules 0.2 μ to 1.5 μ diameter (Fig. 1). The cytoplasm appeared to "glow" with the light refracted by these inclusions, particularly under interferometric microscopy.

Outer Surface: There is a thin lightly-striated pellicle (Fig. 1) which gives a rigidity to the body which resists pressure and deformation. The striations are quite distinct on some individuals, and but barely noticeable on others, even absent or not resolvable on some.

Discussion

A recent, brief report on *Callimastix cyclopis* (Vavra, 1960), indicates that it undergoes a cyclic pattern of development in its hosts. It begins as a globular, unflagellated "larva" in the hemolymph; then becomes flagellate (with no less than two flagella) and escapes from the cephalothoracic cavity of the host. The form with 9 flagella, originally reported by Weissenberg (1912), Vavra (*loc. cit.*) says he rarely saw; but he did find individuals with 3, 4, or 5 flagella, frequently.

Vavra (*loc. cit.*) also states that survival of the flagellates was limited outside the host, in fresh water. They lived but a few hours at low temperature (5° C), and died quickly at "room temperature". This would imply a possible host-dependent relationship, requiring the protozoan to be ingested promptly by another host individual, if the survival is to be continued.

Since the flagellated stage with many flagella (*i. e.* more than 9) is not reported from any copepodian host; and species with more than 9 flagella are reported only from the intestinal tracts of vertebrate herbivores (and here from a molluscan herbivore), it seems possible that the life cycle of the protozoan may involve two hosts, with the copepod harboring larval forms, and another larger herbivore, molluscan or vertebrate, harboring the more adult forms. Such a conclusion requires further study, including infective experiments involving transfer from one host to another potential host, before it may be accepted.

Pending such studies and the results of them, the present reasonable approach is to describe the organism from the snail as a new species, since it differs morphologically from all other described species of *Callimastix*, and is identified in association with an invertebrate host not previously known to harbor any member of the genus.

C. jolepsi n. sp. differs principally in shape, and in number of flagella from other species of the genus. It is egg-shaped with the narrow end bearing the flagella and the cup-shaped depression (the latter is called a "clear zone" by other investigators and writers), whereas *C. cyclopis* is spherical, and *C. frontalis* and *C. equi* have the "clear zone" and the flagella at the broader end of their ovate bodies. The number of flagella borne on the rim of the cup-shaped depression by *C. jolepsi* n. sp. (7 to 11, usually 9 or 10) overlaps

the number borne by *C. cyclopis* (2 to 9), but is less than that of *C. frontalis* (12) or of *C. equi* (12 to 15).

Callimastix jolepsi n. sp.

Diagnosis: 11 to 20 μ long, usually 15 to 18 μ; 12 to 16 μ broad diameter; 10 to 13 μ short diameter; egg-shaped in narrower aspect; oval in broader aspect; one broad surface with polar, cup-shaped depression, apical rim of which bears 7 to 11, usually 9 or 10, flagella; flagella 25 to 30 μ long, of basal diameter 0.25 μ, tapering to barely resolvable tips; nucleus spherical, 3 μ diameter with distinct spherical endosome 2 μ diameter; many irregular inclusions 1 to 3 μ diameter, many refractile globules 0.2 to 1.5 μ diameter; other very tiny granules present; no contractile vacuole; pellicular surface thin, lightly striated; inquilinic in the intestine of the freshwater pulmonate snail, *Helisoma duryi* Say, in Florida, at Gainesville.

Summary

1. A species of the protozoan Genus *Callimastix* is reported from the intestinal tract of the snail, *Helisoma duryi*, in Florida.

2. It is compared to other species of the genus, and distinguished from them morphologically.

3. It is designated a new species, named *Callimastix jolepsi* n. sp., and is described and depicted in detail. A diagnosis is given for it.

4. The possibility of a life cycle for the protozoan, involving perhaps more than one host in the cycle, is discussed.

5. *Callimastix jolepsi* n. sp. is the first species in the genus to be recorded as inquilinic in the digestive tract of a mollusk.

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